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# Research article

Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers fed a high-grain diet<sup>†</sup>

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#### ARTICLE INFO

#### Article history: Received 8 June 2009 Received in revised form 18 May 2010 Accepted 20 May 2010

Keywords:
Average daily gain
Beef cattle
Condensed tannin
Hydrolysable tannin
Methane
Volatile fatty acid

#### ABSTRACT

The objective of this study, which was part of an integrated project to investigate the antimicrobial effects of dietary tannins on native food borne pathogens in beef cattle, was to examine the effects of source of tannin (condensed, CT, vs. hydrolysable, HT) on performance, feed efficiency, ruminal fermentation parameters, and carcass and non-carcass traits in finishing beef steers. Thirty-six crossbred steers averaging  $414 \pm 40 \,\mathrm{kg}$  BW were stratified by initial BW and randomly assigned to one of three treatments: control (CN), CT, or HT tannins. Commercially available tannin extracts were added to a high-grain diet (ME = 11.9 MJ/kg DM) at 14.9 g/kg DM. Mimosa and chestnut extracts provided condensed tannin and hydrolysable tannin, respectively. Steers were individually fed using Calan gate feeders a high-grain diet. Rumen fluid was collected on days 0, 21, and 42 via stomach tube and analyzed for VFA and in vitro methane producing activity. Cattle were harvested at the end of the study and carcass data collected 24-h postharvest. There was no effect (P>0.05) of tannin supplementation on animal performance, ruminal fermentation parameters, in vitro methane producing activity, or carcass and non-carcass traits, except for HCW, EBW, and rumen mass and empty GIT (g/kg EBW). Condensed tannin steers had 3.7% lower (P<0.05) HCW compared to CN with HT steers having intermediate HCW. Hydrolysable tannin treated steers had 2.8% lower (P<0.05) EBW compared to CN while CT steers had intermediate EBW; CT treated steers also had 15.2% higher (P<0.05) rumen mass (g/kg EBW) compared to HT with CN steers being intermediate. This resulted in a 10.2% increase (P<0.05) in total empty GIT (g/kg EBW) for HT steers compared to CT steers with CN steers

Abbreviations: A:P, acetate to propionate ratio; ADG, average daily gain; BW, body weight; CN, control; CP, crude protein; CSH, cottonseed hulls; CSM, cottonseed meal; CT, condensed tannin; DM, dry matter; DMI, dry matter intake; EBW, empty body weight; FBW, final 42-d BW; FCR, feed conversion ratio; FT, fat thickness; G:F, gain to feed ratio; GE, gross energy; GIT, gastrointestinal weight; HBW, BW at harvest; HCW, hot carcass weight; HT, hydrolysable tannin; IBW, initial BW; KPH, kidney pelvic and heart fat; ME, metabolizable energy; NDF, neutral detergent fiber; PRP, proline-rich salivary proteins; REA, ribeye area; RFI, residual feed intake; TDN, total digestible nutrients; TM, trace mineral; VFA, volatile fatty acid; YG, yield grade.

<sup>†</sup> This project was funded (or funded in part), by beef and veal producers and importers through their \$1-per-head checkoff and was produced for the Cattlemen's Beef Board and state beef councils by the National Cattlemen's Beef Association.

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bring intermediate. There was a treatment  $\times$  day interaction for butyrate concentration. For steers fed CT, there was a linear increase in butyrate while the HT steers remained relatively stable and the control steers had numerically lower butyrate. Despite the significant interaction, treatment means on day 42 were not significantly different. Results indicate that neither source of dietary tannin affected performance and feed efficiency. There were no detrimental effects of tannins on other offal measured indicating that tannins supplementation may be a viable option in finishing beef cattle if bactericidal efficacy is established. More research is needed to further our understanding of how tannin supplementation may fit into real-life feedlot situations.

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#### 1. Introduction

Tannins are a complex group of polyphenolic compounds that plants have evolved to deter herbivory (Foley et al., 1999). Tannins are commonly classified as plant secondary compounds (as are oxalates, terpenes, saponins, etc.) and generally have negative effects on animal production. Originally, the term "tannin" was applied to any substance that was able to tan leather; however, currently, it is generally used to denote any naturally occurring substance of high molecular weight which contains a large number of phenolic hydroxylic groups to enable it to form effective cross-links with proteins (Swain, 1979).

Tannins are classified into two broad categories: hydrolysable and condensed (HT and CT, respectively). Hydrolysable tannins consist of a carbohydrate core with phenolic carboxylic acids bound by ester linkage; condensed tannins consist of oligomers of flavon-3-ols and related flavanol residues (Mueller-Harvey and McAllan, 1992). Tannins are ubiquitous in nature, and are widely found in feedstuffs, forages, fodders, and agroindustrial wastes. Dietary tannins have been shown to decrease intake, growth, and caused damage to the gastrointestinal tract in mammals (McLeod, 1974; Robbins et al., 1991; Hervas et al., 2003). Numerous studies have been conducted to examine the toxicity effects of HT with sparse information on CT toxicity (Hervas et al., 2003). The antimicrobial/bacteriostatic activity of tannins may make it useful as a novel agent to control food borne pathogens, such as *Escherichia coli* (Henis et al., 1964; Min et al., 2008). The data presented here is part of an integrated project that seeks to evaluate the use of dietary tannins as a cost-effective pre-harvest strategy to mitigate food borne pathogens in beef production systems.

There is evidence that dietary tannin positively affect ADG in steers grazing winter wheat (Min et al., 2006) and *in vitro* methane producing activity (Min et al., 2005, 2006). Tannins have also been shown to decrease bloat in wheat grazed steers (Min et al., 2006), as well as to decrease ruminal degradability of CP, increasing the amount of CP that reaches the abomasum and small intestine (Teferedegne, 2000). However, there are few studies that have examined the effects of tannins on animal performance while fed high-grain diets. Therefore, the objectives of this study were to quantify the effect of added dietary CT and HT on animal performance, ruminal fermentation parameters, and carcass and non-carcass traits in finishing beef cattle when fed a high-grain diet.

# 2. Materials and methods

# 2.1. Animals and diets

Approval for care and use of animals used in this study was obtained from the Institutional Care and Use Committee of Texas A&M University. Thirty-six crossbred steers (initial BW  $414\pm40$  kg; initial backfat depth  $0.46\pm0.03$  cm) were stratified by initial BW and randomly assigned to one of three treatments: control (CN), mimosa tannin (condensed tannin; CT), and chestnut tannin (hydrolysable tannin; HT). Commercially available tannin extracts were supplemented in a total mixed ration at 14.9 g/kg DM (chestnut tannin: *Castanea sative* Mill; approximately 800 g/kg DM hydrolysable tannins; mimosa tannin: *Acacia mearnsii* black wattle; approximately 700 g/kg DM condensed tannins; Chemtan® Chestnut Powder KPN and Chemtan® Mimosa Powder, Chemtan CO, NH, USA). Originally, tannins were supplemented at 20 mg/kg DM; however, DMI was reduced for several d in the tannin-supplemented calves. Therefore, concentrations of tannins in both treatment groups were reduced to 14.9 mg/kg DM to avoid confounding treatment and DMI. All calves were fed CN diet for 2 d to allow for washout before being fed tannin-treatment diets at 14.9 mg/kg DM for the balance of the experimental period.

Within treatment, steers were assigned to one of two pens (6 steers/pen) and each pen-group randomly assigned to a pen location in the Calan gate facility resulting in two blocks. Each block consisted of one pen from each treatment; the second block was initiated one week following block I to allow for time sensitive sample processing and lab analysis.

Steers were individually fed a high-corn ration (control ration ME = 11.9 MJ/kg DM, tannin rations ME = 11.7 MJ/kg DM; Table 1) for 42 d using Calan gate feeders. The ration was formulated to meet or exceed requirements for medium-frame finishing steers (NRC, 1996). Metabolizable energy was calculated using the Large Ruminant Nutrition System which is based on the CNCPS model described by Fox et al. (2004). Diet ingredient samples were taken weekly and composited for chemical analysis. Steers were acclimated to a high-grain diet using 3 step-up rations over a 30-d period while being trained to the Calan gates. No supplemental tannins were fed during the adaption phase. The 42-d tannin feeding period was selected to simulate a pre-harvest food borne mitigation technology that would be utilized shortly before harvest.

**Table 1** Ingredient and chemical composition of total mixed ration DM basis (g/kg DM).

Ingredient	Treatment diets			
	Control – CN	Chestnut – HT	Mimosa – CT	
Corn	641.1	627.6	627.6	
Hay-sorghum	71.1	71.1	71.1	
CSHa	18.1	18.1	18.1	
CSM <sup>b</sup>	100	100	100	
Molasses	30.2	30.2	30.2	
Limestone	14.9	14.9	14.9	
TM supplement <sup>c</sup>	19.8	19.8	19.8	
Tannin mix	0.0	14.9	14.9	
Chemical composition				
DM	894.9	896.3	896.3	
CP	133.8	132.1	132.1	
NDF	186.1	183.9	183.9	
ME (MJ/kg)	11.9	11.7	11.7	

a Cottonseed hulls.

#### 2.2. Animal performance

Steers were fed their respective diets twice a day (0700 and 1600 h), following a 30-d diet adaption period, and were provided *ad libitum* access to water. During the 42-d feeding period, feed offered was recorded daily and feed refusals and BW measured at 7-d intervals, the treatment rations were mixed and placed into separate feed carts, and feed handling equipment washed in between to prevent cross contamination of tannins. Initial and final BW and ADG were derived from linear regression of BW on days on test for each steer. Feed efficiency was computed as the ratio of gain:feed (G:F). Residual feed intake (RFI) was calculated as the residual of the linear regression of DMI on ADG and BW<sup>0.75</sup> as described by Arthur et al. (2001).

## 2.3. Ruminal fermentation parameters

On days 0, 21, and 42 rumen fluid was collected for VFA, methane, ammonia, and pH analysis. Rumen fluid was collected *via* stomach tube, fitted with a small cylindrical strainer, before the morning feeding, into 50 mL serum vials that were filled to capacity, capped immediately and stored at ambient temperature until analysis later that day. *In vitro* methane producing activity of rumen fluid was determined by *in vitro* incubation of 5 mL rumen fluid mixed with 5 mL anaerobic dilution solution (Bryant and Burkey, 1953) containing 60 mM sodium formate and 0.2 g finely ground alfalfa (to pass a 4 mm screen). The tubes were capped and incubated at 39 °C under a hydrogen:carbon dioxide (50:50) atmosphere. At the end of the 3-h incubation period, methane concentration was determined by gas chromatography according to Allison et al. (1992). For VFA analysis, 1 mL of rumen fluid was diluted 1:10 with water (pH = 7.0) and pH was recorded, samples were centrifuged and the supertnant frozen (-20 °C) for subsequent VFA and ammonia analysis. Ammonia concentrations were analyzed colorimetrically according to Chaney and Marbach (1962). Volatile fatty acids were analyzed *via* gas chromatography (Agilent 6890N, Santa Clara, CA, USA) with a 007 series bonded phase fused silica capillary column (25 m × 0.25 mm × 0.25 mm) and a flame ionizing detector with the following parameters: 1  $\mu$ L injection, injector temperature = 240 °C, oven temperature = 80 °C for 1 min, ramp to 120 °C hold for 5 min, ramp to 165 °C hold for 2 min, detector temperature = 260 °C.

### 2.4. Carcass and non-carcass traits

Upon completion of the study, cattle were randomly allocated to one of four harvest groups to accommodate data collecting activities. Steers were weighed the morning of harvest prior to feeding, and transported to the Rosenthal Meat Science and Technology Center (Texas A&M University, College Station, TX). Dressing percent was calculated as the ratio of hot carcass weight (HCW) to full-body weight taken right before harvest (HBW). Following exsanguination, weights of hot carcass, hide, head, feet and ears, heart, lungs, kidneys, full gastrointestinal (GIT), spleen, liver, and trim (tail, etc.) were recorded. Thereafter, mesenteric fat was trimmed from the reticulorumen, omasum, and abomasums (stomach complex), and the contents removed by rinsing with water prior to being weighed. Fat adhering to the small and large intestines was trimmed and segments of each intestine physically squeezed to remove the contents. Empty body weight was calculated as HBW minus gut fill (Fox et al., 1976). Gut fill was calculated as the weight of the full stomach complex and intestines minus the weight of the emptied stomach complex and intestines. Esophagus, kidneys, and liver were externally and internally examined for lesions. Cold-carcass traits were measured after a 24-h chill. Fat thickness was measured at the 12th rib and ribeye area (REA) was measured between the 12th and 13th rib.

<sup>&</sup>lt;sup>b</sup> Cottonseed meal.

 $<sup>^{\</sup>rm c}$  Trace minerals supplement: guaranteed analysis: 500 mg/kg Co, 2300 mg/kg I, 4000 mg/kg Fe, 1000 mg/kg Se, 4.5 g/kg Cu, 7 g/kg Mn, 19 g/kg Zn (Animal Science Products, Nacogdoches, TX).

**Table 2**Effects of source of tannin on animal performance and feed efficiency traits in finishing steers.

Trait <sup>a</sup>	Treatment	SEM	P-Value		
	$\overline{\text{Control} - \text{CN} (n = 12)}$	Chestnut – HT (n = 12)	Mimosa – CT (n = 12)		
IBW (kg)	412.3	414.4	409.1	12.6	0.95
FBW (kg)	481.3	478.5	479.2	14.2	0.99
ADG (kg)	1.89	1.77	1.92	0.20	0.64
Gain (kg)	79.27	74.43	80.44	8.45	0.64
DMI (kg/d)	12.17	11.40	11.26	0.96	0.50
DMI (g/kg BW)	27.7	26.1	25.8	1.80	0.44
Gain:feed	0.17	0.164	0.177	0.02	0.65
RFI (kg)	0.319	-0.452	0.132	0.58	0.62

<sup>&</sup>lt;sup>a</sup> IBW: initial body weight; FBW: final 42-d body weight; ADG: average daily gain; DMI: dry matter intake; BW: body weight; RFI: residual feed intake.

## 2.5. Statistical analysis

Individual animal was the experimental unit as individual intakes were recorded. Data were analyzed as a completely randomized block design using PROC MIXED of SAS (SAS v 9.1; SAS Inst. Inc., Cary, NC). Additionally, fermentation data utilized the repeated statement. If the interaction term was found not significant it was then omitted from the model, thereby pooled into the residual error term. PROC FREQ with the FISHER option was used to examine the offal necropsy data (scale of 0-3; 0 = normal, 3 = significant abnormality). Significant differences were accepted if P < 0.05.

The model used to analyze fermentation measures was

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha \gamma)_{ik} + e_{ijk}$$

where  $Y_{ijkl}$  = observation,  $\mu$  = population mean,  $\alpha_i$  = treatment effect,  $\beta_j$  = block effect,  $\gamma_k$  = day effect,  $(\alpha \gamma)_{ik}$  = interaction of treatment and day, and  $e_{iik}$  = residual error.

The model used to analyze all other data was

$$Y_{ij} + \mu + \alpha_i + \beta_j + e_{ij}$$

where  $Y_{ij}$  = observation,  $\mu$  = population mean,  $\alpha_i$  = treatment effect,  $\beta_j$  = block effect, and  $e_{ij}$  = residual error; except carcass data which also included a random effect of harvest group.

#### 3. Results

## 3.1. Animal performance

In the current study, the diet offered contained 14.9 g/kg DM of supplemental tannin (HT or CT), which resulted in approximately 168 g tannin consumed per day or 0.38 g tannin/kg BW. Including tannins in the diet at 14.9 g/kg DM resulted in similar DMI for steers in CN and both tannin-treatment groups (Table 2). Additionally, tannin treatments did not affect (*P*>0.05) initial BW, final BW, ADG or feed efficiency traits (G:F, residual feed intake) during the 42-d finishing period.

## 3.2. Ruminal fermentation parameters

There was no effect (P>0.05) of tannin supplementation on rumen pH and ammonia concentrations (Table 3). Added tannins had no effect on the molar proportions of acetate and propionate, as well as the acetate:propionate ratio or the total concentration of VFA. There was, however, a treatment  $\times$  day interaction for butyrate (Fig. 1). For the CT treated calves, there

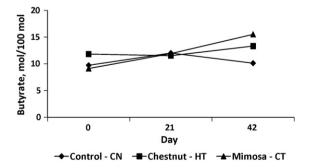


Fig. 1. The interaction of treatment × day for ruminal butyrate molar proportions. Values are expressed as mol/100 mol. (♠) Control diet, no added tannin; (■) chestnut diet, 14.9 g/kg DM added HT; (♠) mimosa diet, 14.9 g/kg DM added CT.

**Table 3** Effects of source of tannin on rumen fermentation parameters (n = 12).

Item/treatment	Day		SEM	P-Value treatment	P-Value day	
	0	21	42			
рН				0.11	0.271	0.280
Control – CN	6.21	6.38	6.37			
Chestnut – HT	6.28	6.24	6.11			
Mimosa – CT	6.25	6.38	6.22			
Methane, µmol/ml fermentation fluid				1.37	0.508	0.008
Control	18.31	21.45	19.44			
Chestnut	15.32	22.16	23.14			
Mimosa	13.72	20.03	20.40			
Ammonia, mg/L				0.52	0.550	< 0.001
Control	1.09	2.67	3.17			
Chestnut	1.49	2.10	2.64			
Mimosa	1.76	2.93	2.54			
Acetate (mol/100 mol)				2.83	0.116	< 0.001
Control	49.47	51.57	55.75			
Chestnut	49.42	51.26	57.71			
Mimosa	45.46	49.88	53.70			
Propionate (mol/100 mol)				5.08	0.311	< 0.001
Control	38.47	34.11	31.79			
Chestnut	36.44	34.84	26.59			
Mimosa	43.03	35.85	28.44			
Butyrate <sup>a</sup> (mol/100 mol)				2.11	0.094	0.006
Control	9.68	11.95	10.08			
Chestnut	11.75	11.52	13.32			
Mimosa	9.13	11.89	15.48			
A:P <sup>b</sup>				0.35	0.367	< 0.001
Control	1.30	1.34	1.8			
Chestnut	1.22	1.29	2.40			
Mimosa	0.88	1.31	1.89			
Total VFA				6.78	0.788	0.394
Control	88.28	85.79	82.56			
Chestnut	83.85	87.50	93.35			
Mimosa	82.23	82.89	93.66			

<sup>&</sup>lt;sup>a</sup> Treatment × day, *P*=0.02; see Fig. 1.

was a linear increase in the molar proportion of butyrate while the HT calves remained relatively stable and the CN calves had numerically lower butyrate concentrations at day 42. Despite the significant interaction, treatment means at day 42 were not significantly different.

## 3.3. Carcass and non-carcass traits

Control calves had higher HCW (*P*<0.05) than CT treated calves with HT treated calves having an intermediate HCW; however, there was no effect (*P*>0.05) of tannin treatment on REA, 12th rib fat thickness, kidney, pelvic, and heart fat (KPH), yield grade, and marbling score (Table 4). Dressing percent was not affected (*P*>0.05) by tannin. Control calves had higher

**Table 4** Effects of source of tannin on selected carcass traits.

Trait <sup>a</sup>	Treatment <sup>b</sup>		SEM	<i>P</i> -Value	
	Control – CN ( <i>n</i> = 12)	Chestnut – HT (n = 12)	Mimosa – CT (n = 12)		
HCW (kg)	286.2b	278.2ab	275.6a	3.97	0.03
12th rib FT (cm)	0.86	0.94	0.91	0.13	0.82
REA (cm <sup>2</sup> )	80.0	76.54	78.2	2.13	0.28
KPH (g/kg HCW)	20.4	17.7	22.0	1.80	0.08
YG	2.70	2.75	2.72	0.10	0.88
Marbling score <sup>c</sup>	5.09	4.88	4.76	0.29	0.53
Dressing (g/kg HBW)	605.8	598.8	593.8	8.90	0.42

<sup>&</sup>lt;sup>a</sup> HCW: hot carcass weight; FT: fat thickness; REA: ribeye area; KPH: kidney, pelvic, and heart fat; YG: yield grade; HBW: body weight at harvest.

<sup>&</sup>lt;sup>b</sup> Acetate:propionate ratio.

b Within a row, means without common letters differ (P<0.05).

<sup>&</sup>lt;sup>c</sup> Slight<sup>00</sup> = 4.00, Small<sup>00</sup> = 5.00 and Modest<sup>00</sup> = 6.00.

**Table 5**Effects of source of tannin on selected visceral organs.

Trait <sup>a</sup>	Treatment <sup>b</sup>			SEM	<i>P</i> -Value
	Control – CN ( <i>n</i> = 12)	Chestnut – HT (n = 12)	Mimosa – CT (n = 12)		
HBW (kg)	472.0	464.9	464.6	3.8	0.32
EBW (kg)	436.1a	424.0b	427.5ab	4.6	0.04
Organs (g/kg EBW)					
Heart	3.87	4.15	3.93	0.16	0.22
Liver	14.9	14.8	14.5	0.52	0.74
Kidney	23.6	2.46	2.38	0.13	0.73
Rumen	28.5a	31.9b	27.7a	1.27	0.01
Small intestine	10.4	10.3	9.98	0.50	0.66
Large intestine	5.54	5.43	5.73	0.38	0.93
GIT dissectible fat	42.6	39.6	39.6	4.40	0.75
Empty GIT	44.5ab	47.6b	43.2a	1.74	0.05
Gut fill	88.1	96.2	86.7	6.45	0.53

<sup>&</sup>lt;sup>a</sup> HBW: body weight at harvest; EBW: empty body weight; GIT: gastrointestinal tract.

(P<0.05) EBW than CT treated calves with EBW intermediate for HT treated calves (Table 5); there was no effect of tannin supplementation on HBW. Empty body weight as a proportion of harvest BW was similar (P>0.05) for all calves (923.2, 912.4, and 920.8  $\pm$  6.2 g/kg HBW for CN, HT, and CT, respectively). Tannin supplementation had no effect on the organ mass as a proportion of EBW for the liver, heart, kidney, small intestine, large intestine, and dissectible GIT fat (Table 5). There was an effect (P<0.01) of tannin supplementation on the proportional mass of the rumen and empty GIT. Calves fed HT had the highest (P=0.01) proportional rumen mass compared to CN and CT treated calves. Additionally, HT treated calves had higher (P=0.05) empty GIT proportional mass compared to CT treated calves, with CN calves being intermediate. There was no effect of tannin supplementation on gut fill; however, HT treated calves had numerically higher proportional gut fill compared to CN and CT treated calves (96.2, 88.1, and 86.7  $\pm$  6.5 g/kg EBW, respectively).

## 4. Discussion

## 4.1. Animal performance

Dietary tannins generally tend to decrease DMI. Hervas et al. (2003) reported that ewes intra-ruminally dosed with Quebracho CT at 3 g/kg BW while fed alfalfa hay had a 95% reduction in DMI after 3 d of dosing. Reduced DMI is thought to be caused by the astringent taste and decreased palatability possibly resulting in food avoidance (Kumar and Singh, 1984). Many mammals, especially browsers, are able to produce proline-rich salivary proteins (PRP) that are able to bind to dietary tannins to inactivate them (Austin et al., 1989). It is the binding of PRP and tannins that produce the astringent taste (Prinz and Lucas, 2000) and subsequent food avoidance. Cattle and sheep are devoid of PRP (Makkar, 2003) so the decrease in DMI due to astringent taste mechanism associated with tannins may not occur in sheep and cattle. However, other proteins are present in the saliva of cattle fed tannin-rich diets which have a high affinity for tannins but are not rich in proline; these salivary proteins tend to form soluble tannin-protein complexes (Makkar, 2003).

There are exceptions to tannin suppression of DMI and in some cases there is an increase in DMI due to tannin supplementation (Woodward et al., 2001; Puchala et al., 2005; Beauchemin et al., 2007). In cattle fed 70% forage ration supplemented with Quebracho CT, Beauchemin et al. (2007) reported no adverse effect on DMI, or ADG. Puchala et al. (2005) reported increased DMI and decreased methane emissions in Angora does fed *Lespedeza cuneata* (CT containing forage) vs. a mixture of *Digitaria ischaemum* and *Festuca arundinacea*. Additionally, late lactation dairy cows consuming *Lotus corniculatus* (CT containing forage) had higher DMI and lower methane per unit milksolids yield compared to cows fed ryegrass silage. Frutos et al. (2004) reported no effect of chestnut HT on DMI and FCR in finishing lambs consuming a high-energy ration (14.2 MJ GE/kg DM). In that study, lambs consumed approximately 0.84 g tannin/kg BW, which is more than double the consumption rate in the current study of approximately 0.38 g tannin/kg BW.

The absence of an effect of tannins on animal performance observed in this study may have been due to the conservative dose of tannins. The primary aim of this study was to evaluate the potential effects of tannins as a bactericidal agent for native food borne pathogens in finishing beef steers. Ultimately, for tannins to be used as a cost-effective pre-harvest strategy to mitigate food borne pathogens, the inclusion rate of tannins cannot have detrimental effects on performance and efficiency.

# 4.2. Ruminal fermentation parameters

Previous studies that have examined the effects of tannins on fermentation parameters in various species have not been consistent. Sheep fed a basal hay diet supplemented with *Elaeis guineense* at 0, 250, or 500 g/kg DM (0, 5, 9 g/kg DM CT; 0, 0.08, 0.14 g tannin/kg BW, respectively) exhibited decreased rumen pH at 5 h post-ingestion and increased ammonia

b Within a row, means without common letters differ (P<0.05).

concentrations; rumen pH began to return to pre-feeding levels after 5 h total VFA concentration increased with 250 g/kg DM supplementation compared to the control diet and acetate concentration increased with *Elaeis guineense* treatment while butyrate concentration increased only in the 250 g/kg DM treatment (Osakwe et al., 2004). In the same study, methane energy losses were found to be lower in sheep fed 500 g/kg DM *Elaeis guineense* diet compared to those fed 0 or 250 g/kg DM *E. guineense*. Makkar et al. (1995) found that both CT and HT tannins decreased VFA production *in vitro* when added at 0.8 mg/mL of medium, but that CT decreased VFA production to a greater extent than HT.

In growing beef cattle fed a forage based diet, Beauchemin et al. (2007), found that supplementation with Quebracho tannin (10 or 20 g/kg DM) decreased the molar proportion of acetate, acetate:propionate ratio, and ruminal ammonia compared to control cattle not supplemented with tannin. The concentration of tannins fed in the current study was approximately 0.38 mg tannin/kg BW, which is very similar to the concentrations used in the study of Beauchemin et al. (2007). In the current study, there was no effect of tannins on VFA. Beauchemin et al. (2007) utilized Quebracho as a source of CT, while in the current study the source of CT used was Mimosa, which suggest that source of CT may influence VFA responses. This inconsistency may simply be attributed to the rumen fluid sampling scheme. Cattle were sampled in the morning before their morning feeding which may have limited ruminal substrate masking any treatment effects. Cattle were allowed *ad libitum* access to their diet, as evidenced by the orts as a proportion of DMI, indicating that the feed bunks contained feed at all times. However, Waghorn and Shelton (1997) fed wethers a diet containing a mixture of fresh cut *L. corniculatus* (37%) and ryegrss/clover for 32 d that contained 10 g CT/kg DM. No effect of CT was observed on VFA concentrations or ammonia in these wethers.

There was no effect of tannin supplementation on *in vitro* methane producing ability (Table 3). Numerous studies have demonstrated that tannins decrease methane production in ruminants (Puchala et al., 2005; Hess et al., 2006; Min et al., 2006). Hess et al. (2006) reported a 13% reduction in methane emission in wethers fed a ryegrass based diet supplemented with 25 g CT/kg DM. Carulla et al. (2005) supplemented wethers at 25 g CT/kg DM consuming a basal diet of ryegrass haylage and found that methane emission was reduced by 13%. However, Beauchemin et al. (2007) reported no effect of CT tannin on enteric methane production in heifers fed a barley silage based diet. The diets fed in these studies were high in forage for which methane emission can be as high as 120 g/kg GE (Johnson and Johnson, 1995). Since a high-grain diet was fed in the current study, much lower methane losses would be expected [20–30 g/kg of diet GE (Johnson and Johnson, 1995)] which may have minimized any potential treatment effect due to tannins. Even a slight decrease in methane production due to tannin supplementation, as evidenced in the literature, would be amplified; as of January 1, 2009, 13.9 million head of cattle were on feed in United States feedlots. A negligible decrease in methane production on a per head basis would translate into much less methane being produced by feedlot animals. The lack of methane response to tannin also reflects the lack of tannin effect on VFA.

The lack of responses to tannin supplementation on ruminal VFA, ammonia, pH and methane producing activity in the current study, could be due to the fact that samples were taken prior to the morning feeding or that they were obtained orally and salivary contamination may have served to dilute VFA concentration. The time of sampling may have also masked any alteration in pH that may have presented itself. Due to sampling time, fermentation substrate may have been limited, however, steers were able to eat at any point they chose as feed was offered *ad libitum*. Orts as a proportion of DMI were in excess of 100 g/kg DM, indicating that calves were fed *ad libitium*. More simply, the lack of tannin effect in the present study may be due to the fact that cattle were fed a high-grain diet.

Research has demonstrated that tannins can also modify microbial populations, which can then alter subsequent diet and nutrient digestibility, VFA profiles, and ammonia concentrations, and ultimately animal performance and feed efficiency. Henis et al. (1964) found an antimicrobial effect of carob pod tannin extract on *Cellvibrio fulvus* (a celluloytic bacterium) *in vitro*; tannin addition also resulted in morphological changes indicating tannin effect on this bacterium. Alteration in gut microbial population was demonstrated in rats, fed CT at 20 mg/kg diet, there was a shift in fecal microbial population favoring *Enterbacteriaceae* and the *Bacteroides* species (Smith and Mackie, 2004). Decreased celluloytic and proteolytic activity was also observed by Tagari et al. (1965) with carob pod extract in an artificial rumen indicating some effect on the microbial population. The lack of effect of tannin supplementation may indicate that ruminal microbial populations were not drastically altered as differences in ruminal fermentation parameters were not observed.

## 4.3. Carcass and non-carcass traits

Frutos et al. (2004) demonstrated no effect of chestnut tannin (HT) on lamb carcass traits when fed a high-grain diet (730 g barley grain and 130 g soybean meal/kg DM; 14.2 MJ GE/kg DM) supplemented with approximately 0.84 g tannin/kg BW; there was no effect of HT supplementation on ADG, feed efficiency, and length of finishing period. They also reported that weights of empty GIT components, skin and non-carcass fat depots did not differ between control and HT treated finished lambs. Additionally, chemical composition of the empty body weight was not different between control and HT treated lambs. Likewise, Kumar et al. (2005) reported that feeding high-tannin sorghum to broilers did not affect carcass traits or proportional weights of visceral organs.

Maxson et al. (1973) demonstrated decreased DM, CP, and TDN digestibilities in steers fed a high-tannin sorghum finishing ration (21.5 g tannin/kg DM) compared to control steers consuming a high-corn finishing ration (5.1 g tannin/kg DM). The authors also reported decreased (P<0.05) ADG in high-tannin sorghum fed steers, numerically higher F:G, and reduced dressing percent, hot carcass weight, and yield grade. This is in partial agreement with the current study as decreased HCW

was observed in CT treated calves; however, in the study reported by Maxson et al. (1973), diet source was confounded with tannin addition.

McBrayer et al. (1983) fed feedlot heifers increasing levels of peanut skins (0, 100, 200 g/kg DM) [high in CT (Stansbury et al., 1950)] to provide 4, 22, and 39 g tannin/kg DM. Heifers fed the diet containing 200 g peanut skin/kg DM had lower ADG and DMI, and reduced DM and CP digestibility compared to heifers fed 0 or 100 g peanut skins/kg DM. In a follow up experiment, ADG, DMI, FCR, and carcass dressing percentage was not affected by the level of dietary inclusion of peanut skins that contained CT 6, 17, or 27 g/kg DM. However, marbling score, yield grade, quality grade, and back fat thickness were higher in steers fed the diet containing 17 g CT/kg DM from peanut skins compared to the control steers, with steers fed the diet containing 27 g CT/kg DM bring intermediate. In both studies, the diet DM digestibilities were lower in animals fed the peanut skin diets compared to the controls.

Tannins can cause toxicity in sheep (Hervas et al., 2003) and cattle (Garg et al., 1992). Hervas et al. (2003) intra-ruminally dosed sheep with 0, 0.38, 1.1, and 2.4 g CT/kg BW consuming a basal diet of alfalfa hay (*Medicago sativa*). No effect of CT was observed in the first three groups; however, the 2.4 g/kg BW group was humanely harvested after 10 d due to lack of DMI and unthriftiness. Many gastrointestinal tract lesions were noted. Garg et al. (1992) described tannin toxicosis in crossbred cattle that consumed immature *Quercus incana* leaves for 2 d. The final mortality due to the consumption was 70%. The HT concentration in the leaves was 97.7 g/kg DM; however, DMI was not quantified so exact HT consumption by the animals is unknown.

In the current study, there was no effect of tannin supplementation on offal and gastric lesion scores of the skin, tongue, esophagus, rumen, reticulum, omasum, abomasum, intestines, liver or kidney (data not shown) at the time of harvest. Offal lesions were not expected as the experimental diet contained well below the amount fed (0.75 mg/kg BW) to sheep that did not result in gastric lesions when fed for 60 d (Frutos et al., 2000).

Collectively, these studies indicate that the responses to tannin supplementation are variable and depend on type, source and concentration of tannin used, animal species, and basal diet fed. Most studies suggest that tannin supplementation at low rates do not have a detrimental effect on economically important traits such as growth efficiency and animal performance.

Condensed tannin treated calves had a 3.7% reduction in HCW compared to CN calves; however, there was no tannin effect on other economically important carcass traits. Presumably, tannin treated calves were able to extract similar amounts of nutrients from their diets to allow similar growth and carcass component accretion. There was no difference in the EBW as a proportion of the harvest BW averaging 918.7 g/kg harvest BW. This is well within the range of 850–950 g EBW/kg BW as described by Owens et al. (1995). The current data indicate that HT calves also had a 2.8% reduction in EBW compared to CN steers. Gut fill was similar across all treatments with HT treated calves having numerically higher gut fill. Condensed tannin treated steers had 15.2% higher rumen mass (g/kg EBW) compared to HT treated steers with CN steers intermediate. This resulted in a 10.2% increase in empty GIT (g/kg EBW) for HT steers compared to CT steers with CN steers being intermediate.

The difference in the proportional rumen mass is interesting, as calves were of similar size and would be expected to have similar gut masses. One possible explanation for this phenomenon is that HT supplies some growth factor that has a positive effect on rumen epithelium causing it to expand in size while CT has the opposite effect; HT can be metabolized in the rumen while CT–protein complexes pass through the gastrointestinal tract with little modification (Makkar et al., 1995). Hydrolysable tannins are largely hydrolyzed in the rumen to acetate and butyrate (Bhat et al., 1998) possibly resulting in slightly larger rumen mass in HT treated steers; however, cell proliferation or VFA uptake by the rumen wall was not measured.

Tannin supplementation offers other added benefits to the beef industry that may help offset possible decreased HCW. Tannins may serve to impede muscle oxidation during storage serving to increase shelf life of whole muscle products. It has been shown in rats consuming high-tannin sorghum, to have lowered markers of protein oxidation in rat muscle after 6 d of refrigerated storage (Larrain et al., 2007). Du et al. (2002) showed higher  $a^*$  (redness) values in thigh patties after 7 d storage at 4 °C from chickens fed 10% high-tannin sorghum. This antioxidant effect of dietary tannins may serve to improve beef product acceptance by maintained redness and decreased oxidation. Evidence of this work was not found in the literature but may have research merit as tannin supplementation in finishing beef calves may help to meet product stability expectations of the consumer as well as being a "natural" product.

## 5. Conclusions

This study was part of a larger experiment investigating the antimicrobial effects of dietary tannins on native food borne pathogens in finishing cattle. No detrimental effect was observed on animal performance, ruminal fermentation parameters, and non-carcass traits; however, decreased HCW was observed in CT treated calves. This may indicate that there is no obvious reason why tannins cannot be added at low levels in the diet to mitigate pathogens if bactericidal efficacy is established. Attention will need to be paid to inclusion ratios so as to avoid lowered DMI and subsequent animal performance. In this study, DMI was immediately reduced upon supplementing CT and HT at 20 mg/kg diet DM, whereas, diet palatability was restored at 14.9 mg/kg DM. Ultimately, for a feed-through additive to be effective it must be value additive; a supplement that is effective for its purpose but decreases animal performance or detrimentally alters carcass traits will not be accepted by industry. This research indicates that HT and CT supplementation at low levels does not have detrimental effects on animal performance or other economically important traits and would make a good candidate for further research on tannin effects of food borne pathogens.

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